REMARKS

Amendments to the Claims

Claims 1-5 and 7-15 were pending.

Claim 3 has been canceled without prejudice.

Claims 1, 2, 4 and 10-12 have been amended.

Claims 1 and 10-12 have been amended to recite the phrase "wherein the Fisher ratio is determined without the use of a prior probability." Support for this amendment can be found in the Specification, for example, at page 9, lines 27-32.

Claim 1 has been amended to recite "comprising: (i) determining said expression levels or patterns of genes and/or proteins of said human tumor tissues from at least two different grades of tumor; and (ii) selecting a set of genes and/or proteins associated with said at least two different grades of tumor differentiation." Support for this amendment can be found in the Specification, for example, at page 7, line 16 through page 10, line 25.

Claim 2 has been amended to recite "obtained from non-cancerous liver (L0), pre-cancerous liver (L1), well differentiated hepatocellular carcinoma (HCC) (G1), moderately differentiated HCC (G2) and poorly differentiated HCC (G3)." Support for this amendment can be found in the Specification, for example, at page 6, line 32 through page 7, line 15.

Claim 4 has been amended to depend from Claim 2.

Claims 10-12 have been amended to delete the Markush language in step (a). Support for this amendment can be found in the Specification, for example, at page 6, line 32 through page 9, line 9.

Claim 12 has been further amended to recite "method of determining" and "expression levels or patterns of the genes and/or proteins selected in step (c) of an unknown sample whose grade of differentiation is to be determined" to better describe the invention. Support for this amendment can be found in the Specification, for example, at page 10, line 34 through page 11, line 8.

Claim 16 has been added. Support for this claim can be found in the Specification, for example, at page 10, line 34 through page 12, line 20.

Claim 17 has been added. Support for this claim can be found in the Specification, for example, at page 12, line 22 through page 13, line 1.

No new matter has been added. Entry of these amendments is respectfully requested.

Rejection of Claims 1-5 and 7-9 Under 35 U.S.C. § 101

Claims 1-5 and 7-9 have been rejected under 35 U.S.C. § 101 as being non-statutory subject matter. In the Office Action, the Examiner suggested that the rejection would be obviated if independent claim 1 is amended to recite an active method comprising "determining the expression levels or patterns of genes and/or proteins by performing an assay for the gene and/or protein levels and patterns" (Office Action at page 4, first paragraph).

As suggested, independent Claim 1 has been amended to recite the steps of method for defining the differentiation grade of a tumor as shown above, rendering the rejection moot.

Rejection of Claims 1-11 Under 35 U.S.C. § 103(a)

Claims 1-11 have been rejected under 35 U.S.C. § 103(a) as being unpatentable over Okabe *et al.* (Okabe *et al.*, *Cancer Research*, March 2001, 61:2129-2173; hereinafter, "Okabe") in view of Adorjan *et al.* (U.S. Publication No.: 2002/0192686 A1; hereinafter, "Adorjan").

Okabe teaches a method of analyzing genome-wide gene expression profile in human HCC using cDNA microarray. By using the Mann-Whitney statistical method, Okabe identified a set of genes that are associated with the HCC progression from the early stage to the later stages of HCC (*see* Okabe, bridging paragraph between pages 2136 and 2137). Okabe classified the HCC progression between two specific HCC groups: (1) the well-differentiated tumor group (*i.e.*, Edmondson grade I; the early stage) and (2) the moderately-to-poorly differentiated tumor group (*i.e.*, the combined group of Edmondson grade II and III; the late stage) (*see* Okabe at page 2136, right col., final paragraph; and Figure 3A). Okabe does not teach methods of the Fisher analysis or selecting genes in descending order of the Fisher ratio where the Fisher ratio are from a comparison between non-cancerous liver and pre-cancerous liver, pre-cancerous liver and well differentiated HCC, well-differentiated HCC and moderately differentiated HCC, and moderately differentiated HCC and poorly differentiated HCC.

Adorjan teaches a method of identifying an epigenetic feature (*i.e.*, DNA methylation between two particular types of cancer: acute myeloid leukemia (AML) and acute lymphobalastic leukemia (ALL). Adorjan teaches the use of the Fisher criterion for determining the degree of separation from one class to another. Adorjan was mainly provided for the teachings directed to the Fisher criterion.

Applicants maintain the arguments submitted in the previous response filed on February 2, 2010 and further assert the following in response to the Office Action dated March 10, 2010. Applicants previously argued that a *prima facie* case of obviousness has not been established because: (1) the combined references cited do not teach or suggest an *in vitro* method of defining the differentiation grade of a tumor into five different classes; and (2) one of ordinary skill in the art would not have been motivated to combine the teachings of Okabe with the teachings of Adorjan; and (3) there is no reasonable expectation of success in arriving at the present invention.

The Examiner stated that: "The combined methods do use genes that are differentially expressed between well differentiated HCC and moderately differentiated HCC (see pages Figure 3 and pages 2136-2137)" (Office Action at page 8, first full paragraph). Contrary to the Examiner's statement, Okabe teaches as follows:

> As in the multistep model of adenoma-to-carcinoma sequence accepted for colorectal tumors, HCCs are considered to develop as well-differentiated tumors and then progress to moderately-to-poorly differentiated states. A comparison of expression profiles between well-differentiated tumors (Edmondson grade I; n=7) and moderately to poorly differentiated tumors (Edmondson grade II or III; n= 13; Fig. 3A) by means of Mann-Whitney test identified a total of 321 genes (including 193 ESTs) that showed different expression patterns between the two histologically divided groups. In addition to the genes encoding liver-specific proteins, they included genes associated with apoptosis and the immune system. Apoptosis-related genes…were preferentially reduced in moderately-to-poorly differentiated tumor, implying that a reduced rate of apoptosis is a major characteristic of tumor progression. (Okabe *et al.*, bridging paragraph between pages 2136 and 2137; emphasis added).

Consistent with Applicants' previous remarks, Okabe divides HCC progression into two states/grades: the early stage (*i.e.*, well differentiated; G1) and the late stage (*i.e.*,moderately-to-poorly differentiated HCC; combined category of G2 and G3). The illustration set forth in Figure 3 of Okabe at page 2135 also confirms Applicants' remarks.

While Okabe merely identifies genes associated with transition from G1 into the combined category of G2 and G3, the present invention is associated with identification of genes that are involved in the transition between G1 and G2; and G2 and G3, among others. Therefore, unlike the statement in the Office Action,[1] Okabe does not teach the use of genes differentially expressed between "well-differentiated HCC (G1) and moderately differentiated HCC (G2)" as in the present invention. Accordingly, the statement in the Office Action is misplaced and does not support the rejection.

In addition, the Examiner also stated that Applicants' arguments are based on the element that is not present in the claims because the claims do not require one to divide HCC into five categories (Office Action, bridging paragraph between pages 8 and 9). Although Applicants disagree, Claims 2 and 10-12 have been amended to reflect that HCC is divided into five grades: L0, L1, G1, G2 and G3. Further, Applicants respectfully assert that expression levels or patterns of the genes and/or proteins are determined in samples from L0 to G3, a set of genes whose expression is significantly associated with each transition can be determined by the descending order of the Fisher ration as set forth in Claim 1. Accordingly, the present invention is directed to a method of defining differentiation grade of a tumor from one stage to another, *e.g.*, between (1) non-cancerous liver (L0) and pre-cancerous liver (L1), (2) pre-cancerous liver (L1) and well differentiated HCC (G1), (3) well differentiated HCC (G1) and moderately differentiated HCC (G2), and (4) moderately differentiated HCC (G2) and poorly differentiated HCC (G3). Using expression levels and patterns of genes and/or protein identified in each transition stage as well as the minimum distance classifier, the present invention enables one to diagnose the differentiation grade of a unknown HCC samples as taught in the Specification at page 10, line

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[1] "The combined methods do use genes that are differentially expressed between well differentiated HCC and moderately differentiated HCC (see pages Figure 3 and pages 2136-2137 of Okabe et al. in particular). Such methods that use genes that are differentially expressed between well differentiated HCC and moderately differentiated HCC are methods that use genes that are differentially expressed between non-cancerous liver and pre-cancerous liver, well differentiated HCC and moderately differentiated HCC, or moderately differentiated HCC and poorly differentiated HCC as required by dependent claim 4 and independent claims 10-12" (Office Action at page 8, first full paragraph; emphasis added)

34 through page 12, line 20. This approach is not taught by Okabe. As discussed above, Okabe does not teach the genes involved in either (3) or (4) above. In fact, Okabe does not teach any set of genes associated with any of the transition stages of (1) through (4) above. Therefore, Applicants' arguments based on the distinctions discussed previously should be duly considered for non-obviousness of the claims, particularly as amended.

Because the use of the Mann-Whitney test in the teachings of Okabe appears to be proper and complete, one of ordinary skill in the art would not have been motivated to look to the teachings of Adorjan to arrive at the present invention. In response, the Examiner stated that: one of ordinary skill in the art would have been motivated because "Okabe et al teaches the importance of analyzing microarray data of tumor states using cluster analysis in order to see how genes are expressed and gain insight into cellular processes involved in various classes of tumor (see page 2137, in particular) and Adorjan et al teaches a Fisher ratio is a 'classical' measure to assess the degree of separation between two classes and the Fisher ratio gives a high ranking for cancer markers where two classes are far apart compared to within class variations (see paragraph 0104-0105, in particular)" (Office Action, bridging paragraph between pages 9 and 10). However, the Examiner's reasoning provides no basis as to why one of ordinary skill in the art would have been motivated to seek out the Fisher ratio taught in Adorjan over the Mann-Whitney test employed by Okabe. As indicated by Okabe, the Mann-Whitney test independently provides sufficient statistical analyses for identifying genes significantly associated with HCC progression from the early stage (G1) to the combined late stage (G2 and G3). Thus, one of ordinary skill in the art reading Okabe would not have been motivated to modify the teachings to substitute the Mann-Whitney test with the Fisher ratio of Adorjan. Applicants respectfully assert that the Office Action is not fully responsive to Applicants' argument and appears to be based on impermissible hindsight.

Further, one would not have been able to arrive at the claimed invention with a reasonable expectation of success absent (1) specific knowledge that HCC progression can be classified into five distinct groups and (2) empirical genome-wide expression data from L0 to G3. The Examiner stated that: "Applicant is arguing limitations not recited in the claims" (Office Action at page 10, first full paragraph). As noted above, Claims 2 and 10-12 have been amended to reflect that HCC progression is divided into five categories and expression levels or

patterns of genes and/or proteins are determined in samples from each of the five grades. Okabe used a combined category of the later stages of HCC termed as "the moderately-to-poorly differentiated tumor group" and made only one comparison between the early stage of HCC (G1; "the well-differentiated tumor group") and the combine category of the late stages (the combined group of G2 and G3; referred to as "the moderately-to-poorly differentiated tumor group"). In contrast, the present application teaches the use of five distinct HCC groups. Accordingly, even if a skilled artisan endeavors to combine the teachings of Okabe with the teachings of Adorjan, one of ordinary skill in the art would not have been able to arrive at the claimed invention with a reasonable expectation of success.

In addition, independent Claims 1, 10, 11 and 12 have been amended to recite "the Fisher ratio is determined without the use of a prior probability." In the formula set forth at page 9, lines 27-32 of the Specification, the coefficient of the denominator is the same (1/2) for every gene and the coefficient of the denominator is also the same (1/2) when any two Grades, such as non-cancerous liver and pre-cancerous liver, pre-cancerous liver and well differentiated hepatocellular carcinoma (HCC), well differentiated HCC and moderately differentiated HCC, and moderately differentiated HCC and poorly differentiated HCC, are compared. Hence, a prior probability (1/2) is eliminated from the formula for calculating the Fisher ratio because the coefficient of the denominator is not dependent on the genes to be evaluated or the grades to be compared.

In sum, a *prima facie* case of obviousness has not been established because one of ordinary skill in the art would not have been motivated to combine or modify the teachings of Okabe and Adorjan to arrive at the claimed invention with a reasonable expectation of success.

The Present Invention Achieves Unexpected Results

Even assuming, *arguendo*, that a *prima facie* case has been established, the *prima facie* case is effectively overcome because the present invention achieves unexpected results. The present invention identifies genes that were not previously identified by the approach used by Okabe. For example, the top 5 genes associated with the transition between G1 and G2 identified by the present invention were not identified by Okabe (*see* Okabe, Figure 3). The genes identified by the present invention are also known to be associated with prostate cancer

tumor progression (*see* the Specification at page 27, line 32 through page 28, line 9). The present invention achieves unexpected results over Okabe which utilizes the Mann-Whitney test.

Rejection of Claims 1-12 Under 35 U.S.C. § 103(a)

Claims 1-12 have been rejected under 35 U.S.C. § 103(a) as being unpatentable over Okabe in view of Adorjan as applied to Claims 1-11 and further in view of Bloch *et al.* (U.S. 6,728,642 B2; hereinafter, "Bloch").

The deficiencies of Okabe and Adorjan are discussed in detail above.

Bloch teaches how to use the "minimum distance classifier" and how to illustrate classified genes into self-organized maps (SOMs).

The teachings of Bloch directed to the use of the minimum distance classifier or SOMs simply do not compensate for the deficiencies of the combined teachings of Okabe and Adorjan.

For the foregoing reasons, Claims 1-12, particularly as amended, are not rendered obvious over the combined teachings of Okabe, Adorjan and Bloch. Reconsideration and withdrawal of the rejection are respectfully requested.

CONCLUSION

In view of the above amendments and remarks, it is believed that all claims are in condition for allowance, and it is respectfully requested that the application be passed to issue. If the Examiner feels that a telephone conference would expedite prosecution of this case, the Examiner is invited to call the undersigned.

Respectfully submitted,

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